NON-CLINICAL MICROBIOLOGY STUDIES TO SUPPORT DEVELOPMENT OF BROAD SPECTRUM THERAPEUTICS

NIAID BROAD SPECTRUM THERAPEUTICS WORKSHOP

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SUSCEPTIBILITY TESTING

- Determine what method(s) best suited for testing agent (see “In Vitro Susceptibility Test Methods” references)
  - Micro broth dilution
  - Agar dilution
  - Disc diffusion
- Incubation environment
  - Aerobic, capnophilic, anaerobic
- Determine effects of medium, plastics, pH, cation concentration, CO_2, surfactants on activity of drug
- Correlate broth dilution MIC with agar dilution MIC

DETERMINE SPECTRUM OF ACTIVITY

- Demonstrate antimicrobial activity against target pathogens
  - 100 of each species relevant to clinical genera and species
  - Recent clinical isolates (within past 3 years)
  - Broad geographical distribution
- Relevant resistance mechanisms (e.g. methicillin resistance, ESBLs)
- Relevant virulence factors (e.g. community-acquired *S. aureus* – PVL gene)

DETERMINE SPECTRUM OF ACTIVITY (cont.)

- Relevant biotypes (e.g. *E. coli* UTI)
- MIC range, MIC_{50}, MIC_{90}
- MBC
- Interaction with biological components (e.g. serum proteins, surfactants)
- Interaction with other antimicrobials
  - Synergism/indifference/antagonism
    - Time kill method vs. checkerboard method (e.g. linezolid + vancomycin)

DETERMINE SPECTRUM OF ACTIVITY (cont.)

- Interaction with other drugs
- If activity of compound is altered by conditions of test which condition will best correlate with efficacy in humans for a particular infection (e.g. addition of supplemental cation increases in vitro activity of drug – efficacy for cSSSI okay but for endocarditis?)

DETERMINE CHARACTERISTICS OF ANTIMICROBIAL

- Postantibiotic effect (PAE)
- Postantibiotic leukocyte effect (PALE)
- Effect of sub-inhibitory concentrations
- Intracellular activity
- Development of resistance
  - Checkerboard method vs. gradient plating
- Effect on production of virulence factors
- Interaction with immune system
- Effect on inflammation (e.g. tetracyclines)

DETERMINE CHARACTERISTICS OF ANTIMICROBIAL (cont.)

- Effect on ecosystems
- Antimicrobial activity of metabolites

TARGET SITE OF ACTION FOR ANTIMICROBIAL

- Systemic versus non-systemic
- Sequestered site (e.g. vegetation, abscess) versus non-sequestered site (e.g. blood, skin)
- Concept of physiological conflict (e.g. aspirin plus antimicrobial, dextranase plus antimicrobial, antimicrobial plus antimicrobial)
- Biofilms
  - Effect of antimicrobial on biofilm
  - Effect of biofilm on antimicrobial

DETERMINE MECHANISM OF ACTION

- Inhibition of cell wall synthesis
- Lysis of cell membrane
- Inhibition of protein synthesis
- Inhibition of DNA and/or RNA synthesis
- Competition with bacterial binding sites
- Other factors to consider
  - Effect on virulence factors (e.g. adherence, hemolysins, toxins)

DETERMINE MECHANISM OF ACTION (cont.)

- Physiological state of bacteria (resting versus replicating)
- Physical state of bacteria (sessile versus planktonic)

DETERMINE MECHANISM(S) OF RESISTANCE

- Intrinsic resistance
  - *Stenotrophomonas maltophilia* resistance to imipenem
- Test organisms with specific mechanisms of resistance
  - Permeability
    - Porins
  - Bacteria with thickened cell wall
    - Vancomycin, daptomycin
  - Enzymatic (e.g. beta-lactamases, extended spectrum beta-lactamases)
  - Drug modifier (e.g. *Enterococcus faecium* – quinupristin/dalfopristin)

DETERMINE MECHANISM(S) OF RESISTANCE (cont.)

  - Ribosomal
    - Changes in affinity of target site (e.g. protein inhibition)
- Ineffective transport (e.g. aminoglycosides - anaerobes)
- Efflux
- Inducible, non-inducible
- Chromosomally linked
- Plasmid linked

DETERMINE MECHANISM(S) OF RESISTANCE (cont.)

- Effect on physiology of cell (e.g. slows growth)
- Structural mutations in preexisting genetic determinants
  - Point mutations
    - Fluoroquinolones – gyrase, topoisomerases
    - Linezolid – point mutation in 23S rRNA several genes high level resistance
    - *Mycobacteria* - streptomycin
- Regulatory mutations in preexisting genetic determinants
  - Decrease the expression of outer membrane porins or increase the expression of multidrug efflux pumps
    - *Pseudomonas aeruginosa* – fluoroquinolones

DETERMINE MECHANISM(S) OF RESISTANCE (cont.)

- Point mutations in acquired resistance genes
- Extended spectrum beta lactamases
- Changes in one or two nucleotides with corresponding changes in amino acids
- Acquisition of foreign DNA
- Transformation, conjugation
- Transposable elements
  - Insertion elements
  - Transposons
    - Tn3, conjugative, composite

DETERMINE MECHANISM(S) OF RESISTANCE (cont.)

- Cross resistance
  - Compare activity to antimicrobials with same mechanism of action
    - Test bacteria with unique mechanisms of resistance
    - MIC range of each group of bacteria
    - MIC_{90}

PHARMACOKINETICS

- Pharmacokinetics
  - Analytical method (balance sensitivity and robustness)
    - Biological versus non-biological - correlation
  - Animal models
    - Factors to take into consideration
      - Species, age, gender, susceptibility to target organisms, circadian rhythm, tolerance to drug
  - Plasma, tissue, infection site (e.g. vegetations – physiology of versus physiology of plasma, tissue)
  - Normal and diseased state
  - Fever

PHARMACOKINETICS (cont.)

- Distribution into infection site – amount, homogeneity, bioavailability
- Active drug versus non-active drug (e.g. protein binding)

PHARMACODYNAMICS

- Pharmacodynamics
- Animal models
- Define in-vivo efficacy parameters
  - AUC/MIC, Peak/MIC, T>MIC
- See “Pharmacodynamic” references

ANIMAL MODELS

- Animal Rule
- Basic screening
  - Mouse protection studies (ex vivo, monoparametric)
- Discriminative animal models of infection (e.g. UTI, foreign body, endocarditis, osteomyelitis)
  - Used to differentiate new agents from related or unrelated agents
- Efficacy when phagocytic system is compromised or inoperative (neutropenic models)
- Synergy or antagonism
  - PD_{50} calculated for combination of agents compared to single agent

ANIMAL MODELS

- In vitro correlation with in vivo results
  - Notorious – in vitro tests fail to predict outcome for device-related infections
- Animal results correlated with human results
  - e.g. efficacy in murine model of pneumonia no efficacy in human pneumonia due to difference in lung surfactants

PRIOR TO INITIATION OF ANIMAL EXPERIMENTS

- Minimum - Determination of MIC and MBC
- In vitro tests should mimic conditions likely to exist in vivo (e.g., pH, pO_2, pCO_2)
- Antimicrobial activity against intracellular organisms determined in growing cells (e.g. *Mycobacterium avium* IJ774A cells)
- Chemostat experiments
- Biofilm experiments (e.g. Robins device)
- PAE, PALE

PRIOR TO INITIATION OF ANIMAL EXPERIMENTS (cont.)

- Formulation
- Ethical issues
- Validation of model
- Experimental design and statistics

ESTABLISHMENT OF IN VITRO SUSCEPTIBILITY TESTING PARAMETERS

- Establish quality control parameters – attempt to develop using organisms currently used (see reference CLSI M-23)
- Establish provisional interpretive criteria prior to clinical studies for specific target pathogens [e.g. *S. aureus*, MRSA, *Streptococcus pyogenes*, *Enterobacteriaceae* (specific genera and species)]
  - Based on activity of drug against target pathogens, PK/PD of drug in relation to infection, results of animal infections

ESTABLISHMENT OF IN VITRO SUSCEPTIBILITY TESTING PARAMETERS (cont.)

- Correlate MIC with disk diffusion zones of inhibition (error-rate bounded method, regression analysis)
- During clinical trials have susceptibility done by dilution method (broth, agar) and disk diffusion testing at central lab
- Analyze results done at local labs with central lab to determine if there are discrepancies – if so determine reason for discrepancy
- Review quality control results from central laboratory concurrently with analysis of susceptibility test results for patient isolates

ESTABLISHMENT OF IN VITRO SUSCEPTIBILITY TESTING PARAMETERS (cont.)

- After clinical trials determine correlation of MIC with clinical and microbiological outcome at “end of therapy” and “test of cure” for specific indications, target pathogens, clinical trial populations
- Make necessary adjustments to MIC
- Correlate MIC with disk diffusion susceptibility results (error rate bounded method)
- Select final breakpoints based on evaluation of PK/PD, overall discrepancy rates, and clinical verification of breakpoints by clinical and bacteriological response (see CLSI M23 reference)

HELP

- We are here to provide “guidance”
  - Come early
  - Come often
  - Come prepared
- Submit protocols before initiating studies
- Consider our recommendations

REFERENCES

- Guidance Documents - One relating to microbiology
  - http://www.fda.gov/cder/guidance/index.htm
- CLSI. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Proposed Guideline. CLSI document M45-P, 2005
- In vitro susceptibility testing methods
  - CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – 7th ed. CLSI document M7-A7. CLSI, 940 West Valley Rd., Suite 1400, Wayne, PA 19087-1898 2006
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  - CLSI. Susceptibility Testing of *Mycobacteria*, *Nocardiae*, and other Aerobic *Actinomycetes*; Approved Standard. CLSI document M24-A, 2003

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